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Protein-Assisted Redox Sensing at Biomimetic Electrode

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I. Executive Summary

Sarnoff is developing a biomimetic protein-assisted redox sensing system based on the organized molecular pathways found in nature. This system makes use of a biomimetic membrane self-assembled on a gold electrode as a bioelectronic interface. This membrane will enable the non-dissipative conversion of a redox event occurring in an aqueous medium to an electrical signal. The non-dissipative conversion is enabled by lipid chains functionalized with prosthetics groups that are organized to mimic concerted, vectorial electron transfer.

In this program, Sarnoff has designed and synthesized electron transfer molecules, and engineered them into an organized self-assembled membrane (SAM). We have demonstrated the vectorial transfer of electrons from a bio-fluid (aqueous) solution to an electrode using this membrane. We also functionalized the SAM with proteins to demonstrate the sensing of redox reaction in aqueous solution.

Major accomplishments during this program are in the following areas:

1. Designed and Synthesized two molecules (GPPT and GPNT) for electron hopping in the self assembled membranes (SAM). Four other more advance molecular designs are also included for future study.
2. Developed a process for a self-assembled membrane on gold, consisting of a matrix of long hydrocarbon molecules (C_{18}) and the electron transfer molecules.
3. Demonstrated vectorial electron transfers across the functionalized SAM, from aqueous solution to the electrode.
4. Functionalized the SAM with redox protein and demonstrate the sensing of redox reaction.

II. Technical Report

This program consists of the following four major tasks as described in our proposal: 1) Electrochemical Cell Design and Setup, 2) Synthesis of Electron Transfer SAM Molecules, 3) Self-Assembly onto Au Electrode, 4) Electrochemical Study of the System. The technical report below is organized to reflect these major efforts.

1. Electrochemical Cell Design and Setup

The vectorial electron transfer mechanism that we are studying is a surface process where the electron is generated by a redox reaction in an aqueous solution, and in turn is driven into the solid-state electrode through the SAM we have designed. This process was examined under dc or ac electrical excitation. To control the surface area of the electrode and the gap distance between electrodes, a standard cell has been designed and fabricated. Shown in Figure 1, the standard cell comprises of a pair of Au electrodes with an active area of 1 cm^2 separated by a 1 cm thick spacer. Each electrode has a 500 nm thick Au film on top of a 50 nm Ti adhesion layer sputtered coated onto an alumina substrate. The spacer is a plastic (Delrin) fixture that supports two electrodes mechanically (nylon screws and nuts). Depending on the experimental requirements, this cell can be submersed into a beaker containing the reaction medium, or inside a spectroscopic optical test cell. In our typical electrochemical experiment, this cell is submersed in the 1 M KCl aqueous solution with a Ag/AgCl electrode as the standard reference.

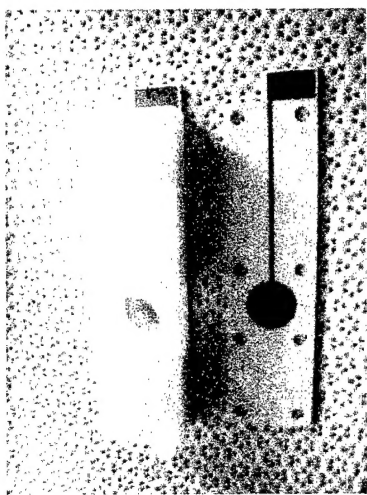


Figure 1. The standard cell is shown with the two electrodes apart. The photo in the right shows a typical experiment setup with the cell submersed in a KCl solution.

To minimize the capacitance and impedance effect of the fluid medium on the electrochemical measurements, we designed and fabricated a thin film cell to significantly reduce the gap between the two testing electrodes. The structure of the cell is shown in Figure 2. In this thin film cell design, two pieces of plastics (Delrin) fixture presses against the electrodes together. A 25 μm thick Teflon plastic sheet (a spacer) separates and electrically insulates the two electrodes. In this Teflon spacer, a rectangular opening of 1 cm^2 is cut and filled with the testing fluid medium, allowing conduction across the SAM electrodes being studied. In this manner, we formed a 1 cm^2 active fluid medium area between two tested electrodes, which are only 25 μm apart. We expect this small gap to significantly reduce the capacitance and impedance effect from the bulk fluid medium in the electrical measurements.

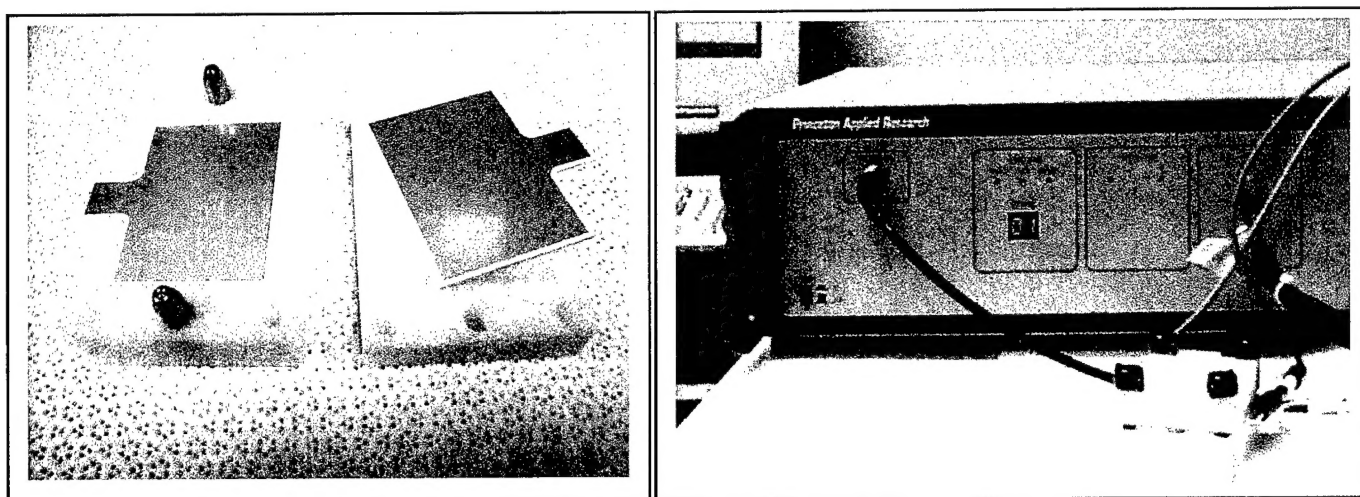


Figure 2. The thin film cell shown with the two electrodes apart. The photo in the right shows a typical experiment setup.

The two main measurement instruments used are a potentiostat (Princeton Applied Research Corporation, Potentiostat/Galvanostat Model 260A) and an impedance analyzer (Hewlett Packard, LF Impedance Analyzer Model 4192A, 5Hz-13MHz frequency range). The potentiostat is controlled by a driver software for electrochemical research (Scribner Associate Inc., CorrWare for Windows). Figure 3 shows the typical experimental setup with the impedance analyzer.

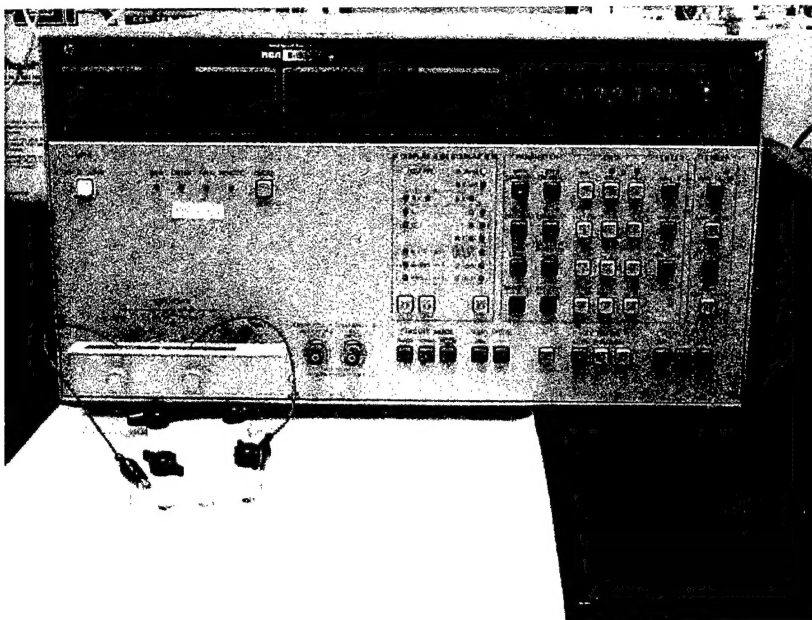
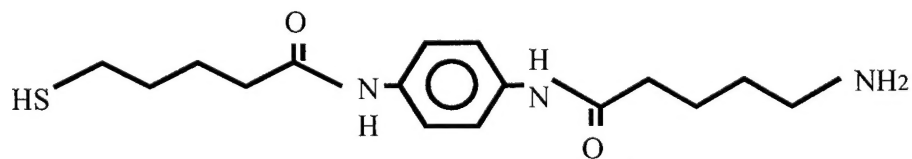


Figure 3. The photo shows a typical impedance analyzer experiment setup with the thin film cell.

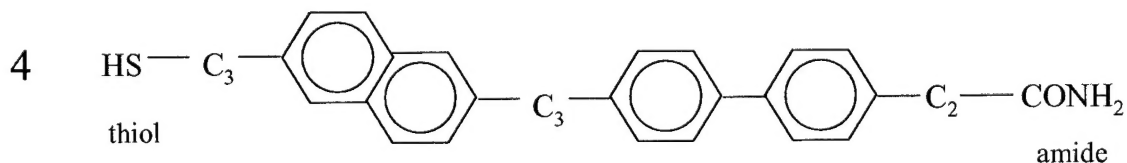
2. Synthesis of Electron Transfer SAM Molecules

The key component for the vectorial electron transfer across the membrane is the electron hopping molecules. The hopping molecules should possess abundant excited energy levels in order to bear the electron for a short, transient period in the process of transport. π -electron rich moieties such as naphthalene and biphenyl are excellent candidates. The molecules are designed to have an axial long chain structure, which can be oriented perpendicular to the electrode surface in the SAM. One end of the molecule is functionalized with a thiol(-SH) group for the interaction to form Au-S bond. The other end of the molecules bear an amine which will be linked to a protein for the later study.

The hopping moieties should have a dipole at an excited state to form an electrical field that can drive the electron toward the thiol (electrode) end. This electronic property ensures that the molecule transfer any electron from the fluid medium to the electrode. We have designed six molecules for electron hopping in self-assembled membranes using the above design considerations. Their structures are listed below.



GPPT



8

methanol. Elemental analysis results indicate that the formula of the molecules match the designs described above. The samples were characterized by ^1H -NMR, UV, FT-IR and elemental analysis. HPLC shows a single specific retaining time for both of the samples.

Characterization data are given below:

GPPT: ^1H -NMR chemical shifts: 7.485, 4.914, 4.859, 3.30, 3.299, 2.51, 2.402, 2.377, 2.356, 2.290, 2.265, 1.972, 1.670, 1.646; UV: 266.5 nm broadband, 202.5 nm broadband. FTIR: 3480, 3440, 3315, 2889, 2867, 1680, 1550 doublet, 1385, 1250 progression, 770, 700 broad, 566 cm^{-1} .

GPNT: ^1H -NMR chemical shifts: 8.02, 8.01, 7.59, 7.57, 4.86, 4.81, 3.31, 3.31, 3.30, 2.63, 2.60, 2.58, 2.56, 2.38, 2.36, 2.35, 2.07, 1.81; UV: 300.5 nm broad, 224 nm strong broad. FT-IR: 3490, 3265, 3220 shoulder, 3112 doublet, 2898, 2865, 1673 doublet, 1585, 1510, 1456, 1412 doublet, 1385, 1256 doublet, 1202, 1089, 1012, 967, 805 cm^{-1} .

The assignments of the chemical shifts and the FT-IR peaks were made to the structural units in the structures of GPPT and GPNT.

3. Self-Assembly onto Au Electrode

All glasswares used in the experiments are cleaned in piranha solution (30% H_2O_2 and 70% H_2SO_4) to reduce organic contaminants. The Delrin fixtures are sonicated in a 200% absolute ethanol for 30 minutes. The nylon screws and nuts are sonicated in acetone for 30 minutes. The Teflon spacers used in the thin film cell were cleaned in piranha solution for approximately 30 minutes, rinsed with DI water for about 45 minutes, and then stored in a 0.05 mM buffer solution (pH 7.0 Trizma pre-set crystals, SIGMA). Also, all final rinse of any cleaning process includes washing with deionized (DI) Water, ethanol rinse, and air dry with N_2 .

The Au electrodes are sonicated in a diluted ChemSolve solution (50 mL of ChemSolve in 1L of DI water) from FISHER for 30 minutes to remove organic contaminants. A two-hour DI water rinse to clean off the ChemSolve follows before the self-assembly process. The cleaned electrodes are stored in a desiccator under Ar atmosphere.

The matrix SAM used is an octadecanthiol molecule (C_{18}) purchased from ALDRICH. To further purify the C_{18} , we dissolved the compound in hexane and filtered it three times with filter paper. The filter solution is air dried for recrystallization and stored in a desiccator. A 1 mM solution of this C_{18} dissolved in ethanol or methanol is

used in our self-assembly process. Both solvents are degassed in N_2 for approximately 40 minutes before use.

GPPT and GPNT were synthesized by Dojindo Inc. The sample crystals were kept in a $-25\text{ }^{\circ}\text{C}$ freezer until use. A 0.25 mM working solution of each of the compounds is made. The solvent for GPPT is N_2 degassed ethanol, while it is N_2 degassed methanol for the GPNT molecule.

Three different types of SAM were formed in our program. The first is a SAM with just 1mM C_{18} dissolved in ethanol. The other two are solutions with various percentages of GPPT and GPNT added to the 1mM C_{18} in ethanol or methanol, respectively. The electrodes are submersed into each solution for approximately 20 hours in room temperature for the complete coating of a SAM. The strong specific interaction between the sulfur atom (from the thiol group) and the Au surface induces the spontaneous assembly of an adsorbed monolayer at the Au-solution interface. So far, SAM with three different concentrations of GPPT (1%, 2.8% and 6.1%) and one (2.8%) concentration of GPNT were fabricated and studied. We have made self-assembled membrane on electrodes in these solutions both in air and under N_2 ambient and found no significant difference.

After the SAM formation, a final rinse as described above is performed and found to be useful in removing unbound molecules on the electrode surfaces. The SAM electrodes and not being studied are placed inside a desiccator under Ar atmosphere.

Control cyclic voltammetric measurements were done to verify the proper coating of the insulating C_{18} SAM. Figure 4 shows a comparison between the blank Au electrode and a C_{18} coated electrode.

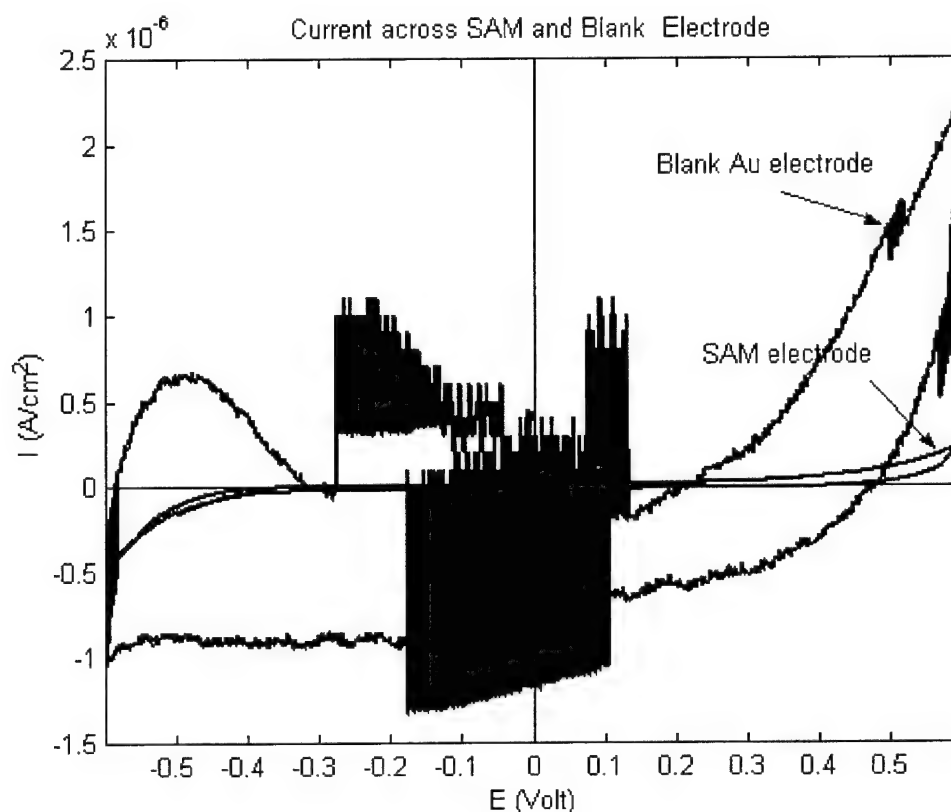


Figure 4. The control cyclic voltammetry measurement comparing a blank Au electrode and a C18 coated electrode. Much noise was observed with the blank electrode, most likely due to random noises from the fluid the triggers the overload circuit in the potentiostat. These noises are minimal in measurements of SAM coated electrodes.

4. Electrochemical Study of the System

4.1 SAM and Hopping SAM

The cyclic voltammograms (CV) of the C₁₈ SAM in both standard cell and thin film cell are shown in Figure 5. The CV data show a typical feature of polarizable electrode, indicating that there is no current flow across the surface in the voltage range from -0.4 volt to 0.4 volt. In the standard cell, a Ag/AgCl electrode was used as the potential reference while no reference was used in the thin film cell. Note that the magnitude of the current recorded here is very small, in the order of 10^{-8} A/cm².

In contrast, the I-V feature of the SAM containing a hopping component, GPPT, shows strong conductivity in the reduction current region (see Figure 6). In the negative voltage range, the hopping SAM surface still to have shows a strong capacitive effect, indicating that the double layer holds. We noted that the contact angle of water drop on the electrode surface was small, characteristic of hydrophobic feature after several cycles of voltage sweeps. Judging from the contact angle of water on the hopping SAM surface

after the CV measurement, the SAM monolayer is stable after experiencing the electrical excitation. This also suggests that the direct contact of Au (the SAM substrate) with aqueous solution is unlikely.

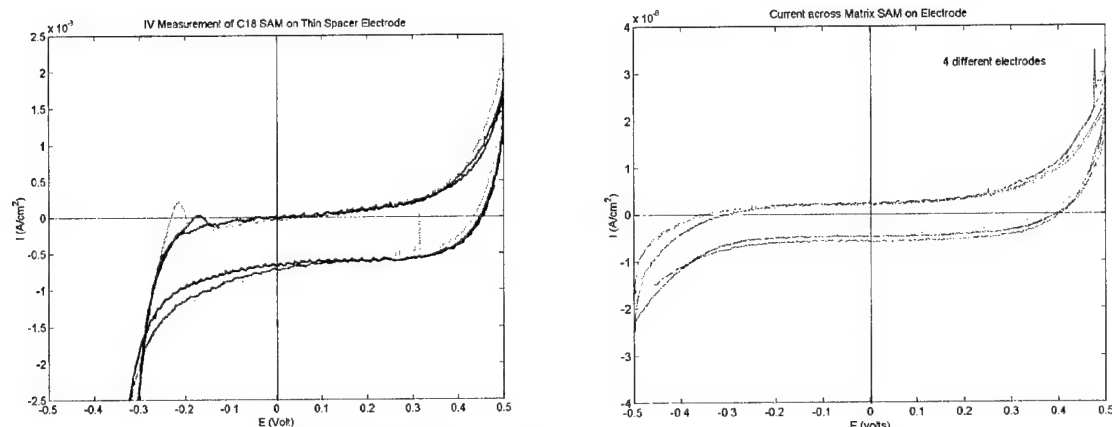


Figure 5. The cyclic voltammograms of the C₁₈ SAM on Au in the standard cell (left) and in the thin film cell (right).

This is the first evidence showing that the hopping SAM surface conducts a vectorial electron transfer, in this case, from the aqueous solution to the solid-state

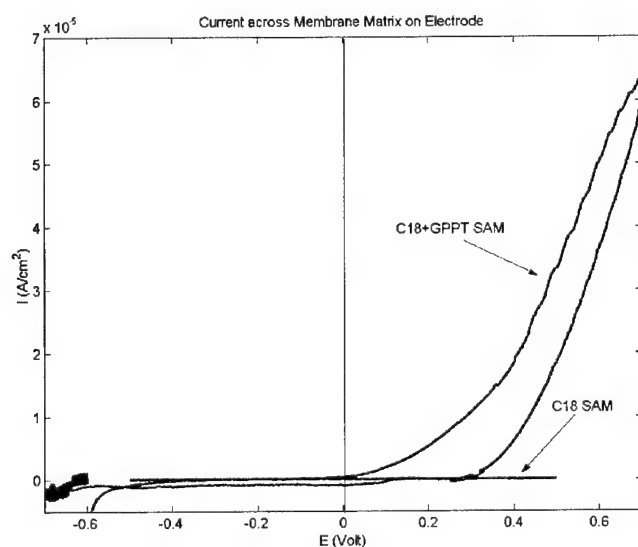


Figure 6 The CV curve of the C₁₈+GPPT SAM in comparison with that of the C₁₈ SAM

electrode. This IV curve is similar to that of solid-state diodes. Apparently the functionalized SAM serves as a diode. Assuming that the 2D concentration of the GPPT in the SAM is the same as that of the membrane forming solution, the surface coverage is estimated to be 2.8 %.

Similar diode behavior is also observed for the C_{18} -GPNT SAM on Au. In this SAM, the naphthalene bearing molecules were coadsorbed with C_{18} onto the Au electrode. Large modification on the IV feature is evident in Figure 7. The current turn-on point shifts toward more negative region than that detected for the C_{18} -GPPT SAM electrode. In the conductive region, from -0.2 to 0.6 volts, the IV plot is almost linear, a typical ohmic conduction. It is noted that the diffusion-caused hysteresis is negligible, i.e. the current difference between forward and backward sweep is very small.

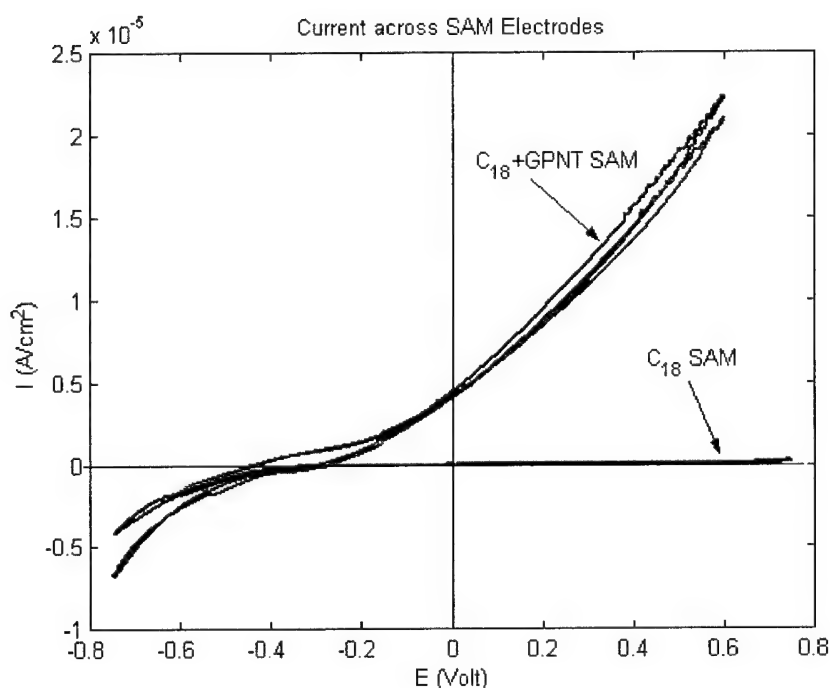


Figure 7 The cyclic voltammograms of C_{18} -GPNT SAM on Au and C_{18} SAM on Au

In order to clarify the conductive nature of the hopping SAM electrode, potentiostatic technique is employed to record the chronoamperometry of the system. In this measurement, the time-dependent current across the hopping SAM electrode is recorded while a constant voltage is applied to the electrode. The measured result is shown in Figure 7.

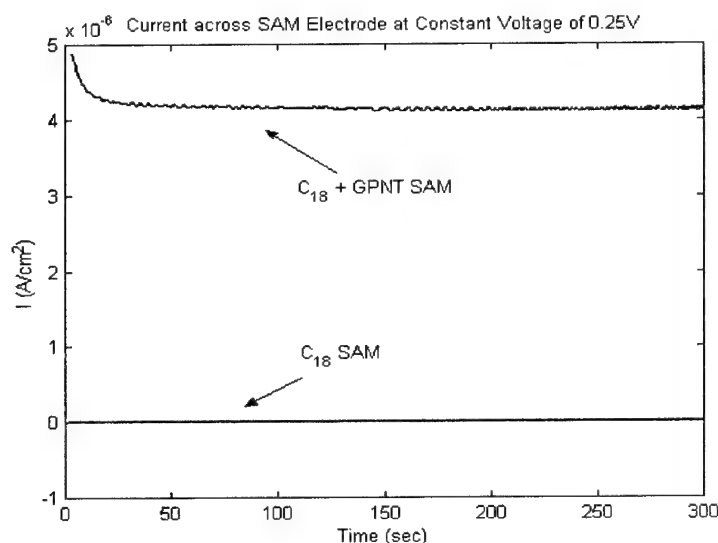


Figure 7. Time dependent current across the SAM with (blue curve) and without (red curve) the hopping molecule, GPNT.

This fact is in good agreement with the CV curve observed in Figure 6. The potentiostat current is also recorded for the C₁₈ SAM electrode (the lower curve in Figure 8). The current value is in the order of 100 nanoamperes at the beginning and decays quickly to a level near zero. The data suggests that the C₁₈ SAM electrode is totally polarizable.

This vectorial electron transfer SAM is used to detect the redox of myoglobin, Mb, a redox protein. The Mb molecules were attached to the outer surface of the C₁₈-GPNT SAM through incubation of the SAM in Mb buffer solution (pH 7.0). After such surface reconstitution, two features are evident in

The current across the C₁₈/GPNT SAM is shown to decay in the first 25 seconds and reach an equilibrium at the level of 4 $\mu\text{A}/\text{cm}^2$. The charge saturation is the typical capacitance behavior and represents the polarization at the surface. However, the conductive feature dominates the current flow.

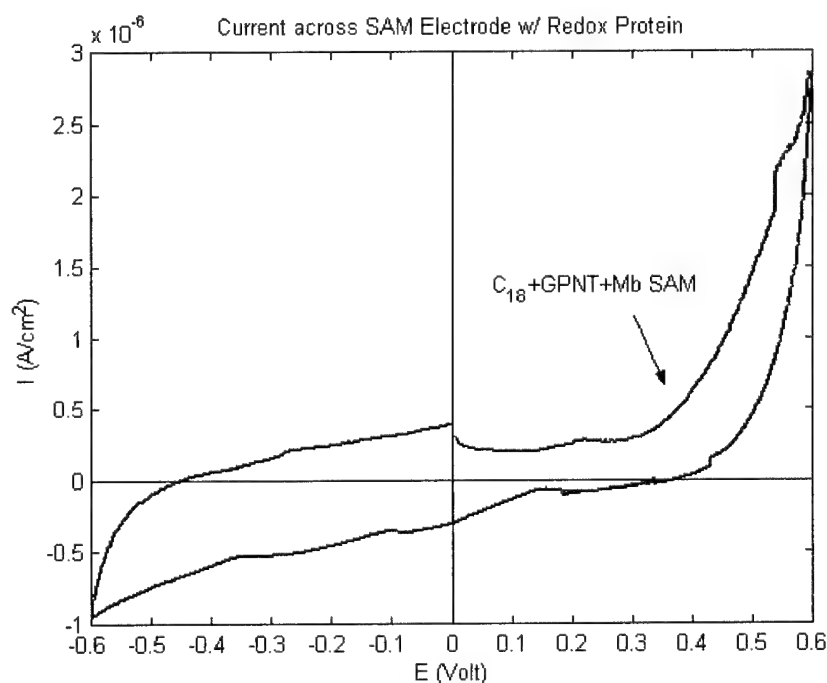
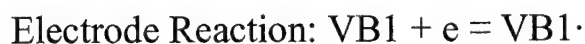
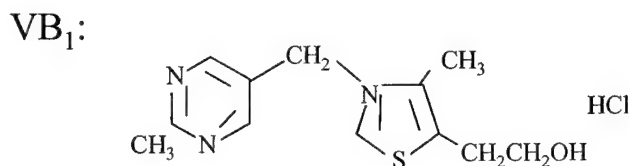


Figure 8. The CV curve of C18-GPNT SAM electrode in an aqueous Mb solution.

the CV data. As shown in Figure 8, the CV curve clearly shows no obvious diode behavior and two reduction waves appear at 0.24 and 0.4-0.5 volts. Also, an oxidation current is seen as a wide wave in the negative voltage region. These redox features are not observable with C₁₈ SAM modified electrode, indicating that the GPNT-functionalized electrode provides a sensitive surface for the Mb redox detection.

4.2 Vitamin B1 Redox Sensing

We chose the redox reaction of vitamin B1 (VB1) in aqueous solution as a model system for the redox sensing. The redox is a single electron transfer process as presented below. The $E^0 = -0.21$ V (vs. HNE). The neutral VB1 is a good electron acceptor as well as an electron donor. It attaches to the Mb with its OH group binding with an amine group on the protein. The electron generated in the redox is considered to transfer across the SAM through the hopping molecules embedded in the SAM.



The cyclic voltammograms of the C₁₈-GPPT SAM electrode in an aqueous solution (pH 7.0) containing VB1 are shown in Figure 9. The voltage sweep starts from 0

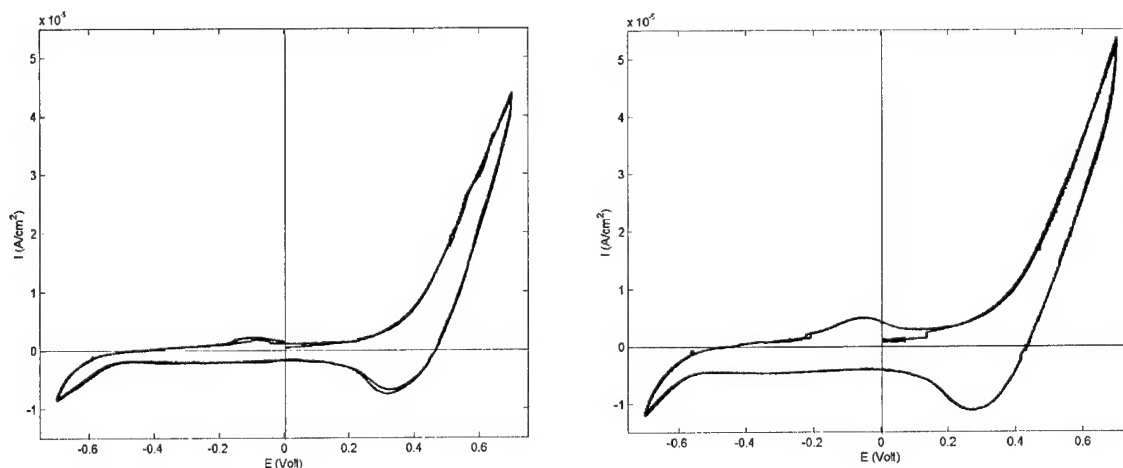


Figure 9. The C18-GPPT SAM electrode detecting the VB1 redox at sweep rates of 40 mV/s and 15 mV/s, respectively.

volt toward positive voltage. An oxidation wave is observed at 0.27 volts first on the potential sweep toward negative potential, being attributable to the electrode process: $VB1 + e = VB1\bullet$. The reverse process is detected on the sweep toward positive potential, a reduction wave at -0.07 volts. The redox waves slightly shift as the sweep rate changes.

The diode feature is observed to dominate in the CV curves in Figure 9. This suggests that, unlike the Mb-binding GPNT SAM (Figure 8), the VB1 does not inhibit the current across the GPPT SAM. The current turn-on potential is shifted to 0.25 volts, in comparison with 0 volt without the VB1 existence (see Figure 8). This implies that there exists a superficial layer of VB1 onto the C_{18} -GPPT SAM.

4.3 Mb-Assisted Redox Sensing by Electrochemical Impedance Spectroscopy

With ac electric excitation, the charge transfer resistance, solution resistance and capacitance can be extracted from Faradiac impedance measurements. The redox system consists of a Au electrode, a C_{18} -GPNT SAM on the Au electrode, a Mb adsorbed layer attaching to the GPNT in the SAM and a thin layer of the aqueous solution containing ionic electrolytes. The entire system is assembled in the thin film electrochemical cell as illustrated in Figure 2. A RC parallel circuit is chosen as the equivalent circuit of the redox system illustrated below.

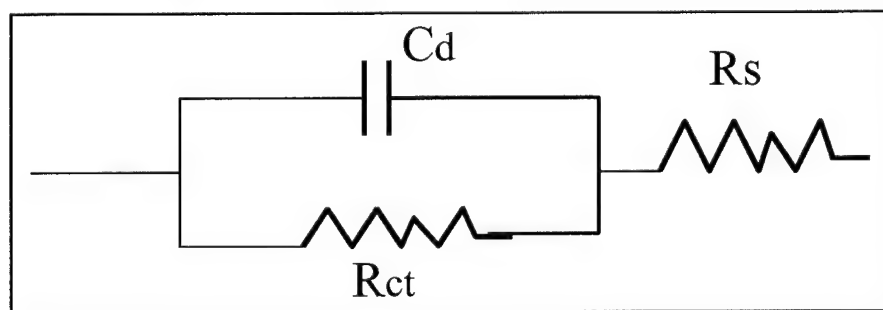


Figure 10 and 11 show typical impedance spectra measured with the designed system. Since the impedance, Z , is expressed by:

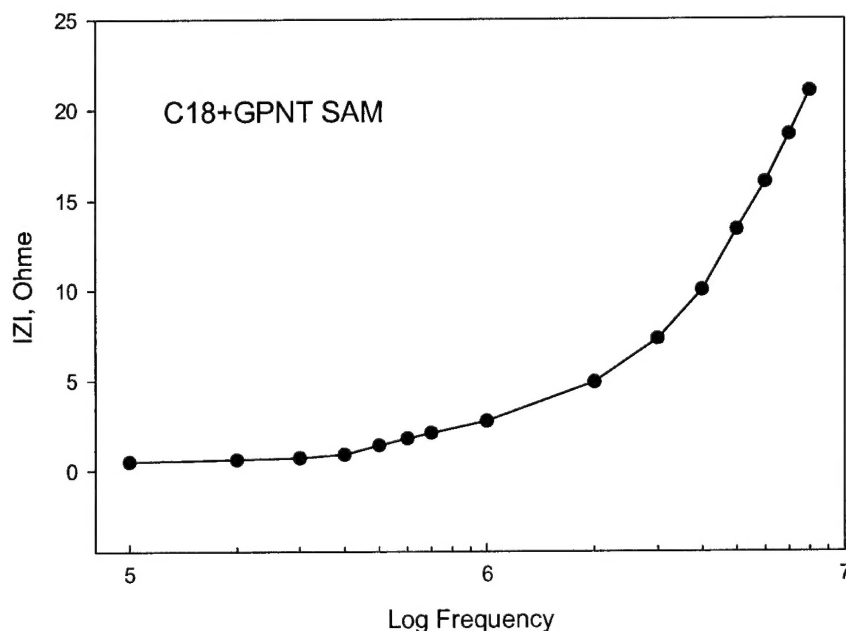
$$Z = Z_{Re} + jZ_{Im} = |Z| (\cos\theta + j\sin\theta) \quad (1)$$

its real part and imaginary part can be calculated from the measured $|Z|$, the magnitude, and θ , the phase angle. The charge transfer resistance, R_{ct} and the solution resistance, R_s , and the double layer capacitance, C_d , are related by the equations:

$$Z_{Im} = Z_{re} - R_s - R_{ct} + 2\sigma^2 C_d \quad \text{for low frequency limit} \quad (2)$$

$$(Z_{Re} - R_s - R_{ct}/2)^2 + Z_{Im}^2 = (R_{ct}/2)^2 \quad \text{for high frequency limit} \quad (3)$$

Thus by measuring the impedance at low frequencies and high frequencies, the charge transfer resistance, R_{ct} , can be extracted from the observed data. The charge transfer



resistance, R_{ct} , for Mb-assisted VB1 redox process on C₁₈-GPNT SAM is calculated, from the measured impedance spectra, to be near zero. This indicate a non-dissipative charge transfer at the modified electrode.

Figure 10. The electrochemical impedance spectrum measured for C18-GPNT SAM at the 30 mV ac excitation.

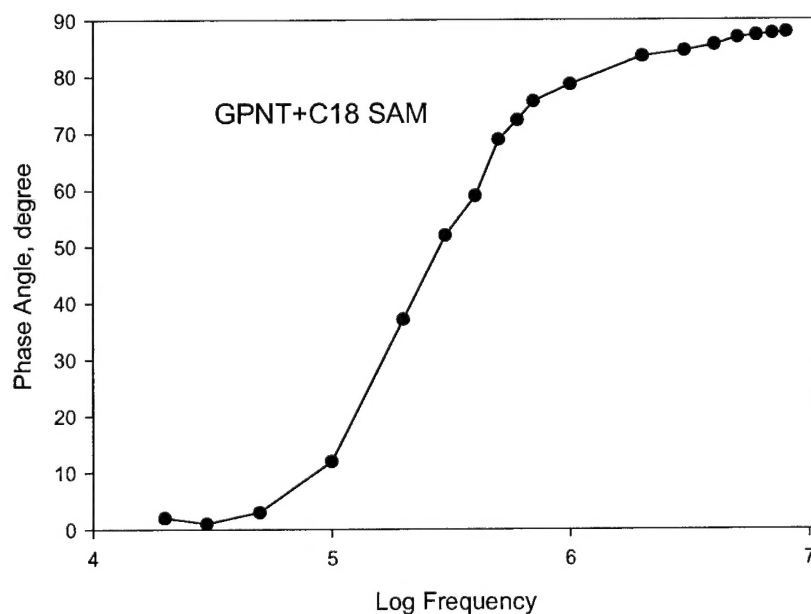


Figure 11. The phase angle versus ac frequency measured for C18-GPNT SAM at the excitation of 30 mV.

5. Conclusion

In this program, we have designed and implemented a biomimetic SAM with vectorial electron transfer molecules. We developed a process to self-assemble these membrane onto a Au electrode. We also functionalized the SAM surfaces with redox protein and studied the ability of SAM to sense the redox reactions. We demonstrated with electrochemical methods that the designed redox sensing system showed vectorial electron transfer functionality.

The electrochemical measurements indicated that the SAM with the designed molecules does have vectorial electron transfer properties and can be used for electrical sensing of redox reactions in aqueous solution. These preliminary results reveal unique properties in the SAM developed in this feasibility program that can be exploited in future technology development. While the electrical properties of the C₁₈ SAM are readily repeatable, the yield for functional SAM with electron transfer molecules is very low. This low yield limits the ability to thoroughly study the properties of the vectorial electron transfer SAM, such as the optimal concentration for efficient electron transfer, the sensitivity to other redox reactions, the stability of the electron transfer molecules, etc. We now believe that the main reason for the low yield is the inability to obtain a reproducible roughness from the sputtered Au surface. One simple improvement will be the use of electrode plating technique for the Au deposition, which can provide a better metal surface. Another method to resolve the roughness issue is the use of a Chemical Mechanical Polishing process on the electrodes after their fabrication. The polishing should provide a more controlled roughness on the Au surface, allowing a more reproducible self-assembled process. We hope to test these ideas in future studies.

While we have not achieved a complete understanding of the biomimetic SAM electrode, we have demonstrated the feasibility of vectorial electron transfer from redox reactions to a metal electrode through the SAM. This biomimetic SAM electrode platform can potentially provide the bioelectronic interface between biomolecular signal pathways to electronic circuitry.

III. Milestone Report

Progress is referenced to milestones listed on the Milestone chart in appendix A1, as described in the original proposal.

All milestones have been successfully reached and all tasks have been accomplished as planned.

A.1 Milestone Chart

ID	Task Name	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
1	Electrochemical Cell Design and Setup								
2	Synthesis of SAM Molecules								
3	Self Assemble to Au Electrode								
4	Characterization Data of SAM								
5	Electrochemical Study of the System								
6	Final Report								

A.2. Financial Report

Name of Contractor: **SARNOFF CORPORATION**
 Financial for Date: **August 15, 2002 thru February 8, 2003**
 Contract Title: **Protein-Assisted Redox Sensing at Biomimetic Electrode**
 Contract No: **F49620-02-C-0082**
 Period of Contract: **August 15, 2002 thru February 14, 2003**

CONTRACT SUMMARY:	Cost	Fee	Total
Initial Contract Value:	\$216,336	\$16,442	\$232,778
Remaining to be funded:	\$0	\$0	\$0

MAN-HOURS:

LABOR	773
MANAGEMENT	26
Workhours Remaining	35
% Workhours to Date	96%

Labor Costs:	\$123,655
Management Costs:	\$4251
Travel Costs:	\$2073
Other Costs:	\$74,912
*Current Period Expenditures:	\$204,891

FUNDS:

	Cost	Fee	Total
Cumulative Total Spent to Date	\$204,891	\$16,442	\$221,333
Open PO Commitments	\$11,445	\$0	\$11,445
Remaining funded value:	\$0	\$0	\$0

WORK COMPLETION:

Percentage of Total Contract Value Expended and obligated to Date	96%
Percentage of Funds Expended and obligated to Date	100%
Percentage of Funded Work Completed to date	100%

*note: does not include fixed fee of \$16,442